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16. ABSTRACT

The separate and combined effects of protein deprivation and nitrofen exposure were studied in the pregnant rat. Animals were fed diets containing 24, 8, 6 or 4% casein throughout gestation. Within each diet group, subgroups were gavage-fed with 12.5 (lower dose) and 25 (higher dose) mg nitrofen/kg body weight or with oil carrier only on days 7–21 of gestation. Dams were weighed and food intake was measured daily. On day 21 of gestation, cesarean-derived pups were examined for congenital anomalies and dissected for determination of organ weights. Skeletons were alizarin-stained and examined for skeletal anomalies and developmental stage. No effects on the skeleton or gross congenital anomalies were seen. Fetal size and weights of liver, kidney, intestine, heart, lung and brain were reduced with decreasing casein content of the diet and as a result of the higher dose of nitrofen. An effect of interaction between diet and nitrofen exposure was shown in the kidney, intestine and lung weights. Specific toxicity affecting organ size was shown to occur in the intestine and lung. An interaction between diet and nitrofen specifically affected kidney and intestine. Brain size tended to be preserved, a possible protective mechanism negated by nitrofen exposure. The data suggest also that kidney, intestine and lung are particularly affected. Effects, however, occur primarily in young of severely malnourished dams receiving a relatively high dose of nitrofen.

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EFFECTS ON THE FETUS OF MATERNAL NITROFEN EXPOSURE IN THE PROTEIN-DEPRIVED RAT*

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SUMMARY

The separate and combined effects of protein deprivation and nitrofen exposure were studied in the pregnant rat. Animals were fed diets containing 24, 8, 6 or 4% casein throughout gestation. Within each diet group, subgroups were gavage-fed with 12.5 (lower dose) and 25 (higher dose) mg nitrofen/kg body weight or with oil carrier only on days 7—21 of gestation. Dams were weighed and food intake was measured daily. On day 21 of gestation, cesarean-derived pups were examined for congenital anomalies and dissected for determination of organ weights. Skeletons were alizarin-stained and examined for skeletal anomalies and developmental stage. No effects on the skeleton or gross congenital anomalies were seen. Fetal size and weights of liver, kidney, intestine, heart, lung and brain were reduced with decreasing casein content of the diet and as a result of the higher dose of nitrofen. An effect of interaction between diet and nitrofen exposure was shown in the kidney, intestine and lung weights. Specific toxicity affecting organ size was shown to occur in the intestine and lung. An interaction between diet and nitrofen specifically affected kidney and intestine. Brain size tended to be preserved, a possible protective mechanism negated by nitrofen exposure. The data suggest also that kidney, intestine and lung are particularly affected. Effects, however, occur primarily in young of severely malnourished dams receiving a relatively high dose of nitrofen.

*This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and reviewed for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. *Address all correspondence to:* Dr. Frances J. Zeman, Dept. of Nutrition, University of California, Davis, CA 95616, U.S.A.

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Key words: Nitrofen; 2,4-Dichlorophenyl-*p*-nitrophenyl ether; Protein deficiency; Pregnancy; Fetus

INTRODUCTION

Nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether) is a pre-emergence herbicide which is relatively non-toxic in adult rats and mice. It has an acute oral $LD_{50} > 2600$ mg/kg [1]. In the case of exposure to nitrofen during pregnancy, however, the material may be more toxic. Decreased pup survival has been reported to occur at dietary levels of 1000 ppm [1,2]. Doses of 12.5 mg/kg/day on days 8—16 of gestation [3] and of 31.2—50 mg/kg/day on days 8—18 of gestation also were stated to reduce pup survival [4]. The LD_{50} was 116 mg/kg/day with day 11 as the most sensitive day in the rat [5]. In the mouse, the lowest dose level affecting pup survival to postnatal day 3 was 50 mg/kg/day on days 7—17 [6]. Abnormalities seen in newborn rat pups include cardiac abnormalities, diaphragmatic hernias and retarded lung development [4,5]. Exposure to nitrofen has also been shown to alter harderian gland development and renal morphological and functional development [7—9]. It might be used, therefore, as a prototype for the study of the effects on the fetus of the diphenyl ethers.

Protein deficiency during pregnancy also has many effects. About 60—75% of female rats fed a protein-restricted (6%) diet during pregnancy fail to litter. Since the diet restriction began only after mating, it appears that embryos fail to implant or are resorbed [10]. Young from those litters which are produced have a much higher mortality rate postnatally [10]. These young also had reduced body weight and length. Liver and kidney weights were reduced even more than was body weight [10]. Kidney morphology was delayed and function was compromised [11,12] as was the morphology and function of the digestive tract and pancreas [13—16].

The possible interactions of protein deficiency and nitrofen toxicity in pregnancy has not been investigated. The present study was undertaken to compare the separate and combined effects of various levels of protein deficiency and various levels of chronic exposure to nitrofen.

METHODS

Virgin, female Sprague—Dawley rats (Bantin and Kingman, Fremont, CA) were housed singly in stainless steel wire-bottom cages and fed, before mating, with a pelleted rat feed (Purina Chow, St. Louis, MO). Tap water was available ad lib. Room temperature was 22 ± 1°C, and a 20 h light/14 h dark cycle was maintained. When females weighed 180—200 g, they were mated with normal proven males on a one-to-one basis. Mating was confirmed by the presence of copulation plugs. The day following the night of mating was designated as Day 0 of pregnancy.

Throughout pregnancy, rats were fed, beginning on Day 0 of pregnancy, a

semi-purified diet containing 24% (control), 8%, 6%, or 4% (protein-restricted) casein as the sole source of protein. The protein restriction levels were chosen to vary from marginal to severe but still supporting production of live young. Dextrose was added to the protein-restricted diets to make them isocaloric. The composition of the control diet is shown in Table I.

Each diet group was divided into 3 subgroups. One subgroup in each set was gavage-fed 0.5 ml of oil (Mazola, Best Foods, Englewood Cliffs, NJ) carrier only on days 7—21 of gestation. The other 2 subgroups were gavage-fed 12.5 or 25.0 mg nitrofen (technical grade, supplied by U.S. Environmental Protection Agency) in 0.5 ml of corn oil/kg body weight, also on days 7—21 of gestation. The 12.5 mg level is that which is considered a marginal or moderate dose, while the higher dose was the level at which there are few survivors during postnatal life although live litters are produced [4].

Females were weighed and food intake was measured daily. Daily doses of nitrofen were based on the body weight for that day. Feed efficiency, grams of weight gain per gram of feed intake, was calculated from feed intake and weight gain.

On day 21.5 of gestation, young were taken from anesthetized females by cesarean section and the following were noted for all young: number in

TABLE I

COMPOSITION OF DIET

	Control (%)
Glucose[a]	60
Casein[b]	24
Corn oil[c]	8
Salt mix[d]	6
Vitamin mix[e]	2
BHT	0.02

[a] Cerelose, Corn Products Co. (CPC International), New York, NY.
[b] ICN Nutritional Biochemical Co., Cleveland, OH.
[c] Mazola oil®, Best Foods International, Inc., Englewood Cliffs, NJ.
[d] Expressed as g/100 g of salt mix: $CaCO_3$, 15.5; Ca_2HPO_4, 41.64; K_2HPO_4, 29.48; NaCl, 4.69; $MgSO_4$, 7.296; $CrK(SO_4)_2 \cdot 12H_2O$, 0.01061; $CuSO_4 \cdot 5H_2O$, 0.07240, KI, 0.00072; $FeSO_4 \cdot 7H_2O$, 0.64487; $MnSO_4 \cdot H_2O$, 0.56563; $ZnCO_3$, 0.08475; Na_2SeO_3, 0.00805; $Na_2MoO_4 \cdot H_2O$, 0.02948; and $KF \cdot 2H_2O$, 0.01842.
[e] Expressed as g/100 g of mix (except as noted): inositol, 2.5; ascorbic acid, 0.5; Ca-pantothenate, 250 mg; thiamin · HCl, 150 mg; pyridoxine · HCl, 150 mg; nicotinic acid, 150 mg; menadione, 125 mg; riboflavin, 50 mg; *p*-aminobenzoic acid, 50 mg; biotin, 1.25 mg; folic acid, 3 mg; vitamin E (Rovimix E-50%), 1.19; vitamin A (Rovimix A-250), 273 mg; cholecalciferol (Rovimix-AD, 325/325), 23 mg; vitamin B-12 (Merck 12 + mannitol), 15 mg; glucose, 89.5; and choline · Cl (70% solution), 7.15. (Rovimix, Hoffman-LaRoche, Nutley, NJ; Merck & Co., Rahway, NJ.)

the litter and location of each pup in the uterus, fetal and placental weights, sex, and crown-rump length. Fetal to placental weight ratios and fetal weight to length ratios were calculated.

Following decapitation of the pups, weights of liver, kidney, intestine, heart, lung, and brain were obtained from 3 pups chosen at random from each litter. Organ weight-body weight ratios were calculated. The skeletons of 3 additional young were stained with alizarin red and examined for presence or absence of ossification sites in the axial and appendicular skeletons. In addition, skull length, spine length, pelvic length, ilium length, bi-iliac width and bi-acetabular width were made from X-ray photographs of alizarin-stained skeletons.

All pups were examined externally for gross congenital anomalies. Three to 6 pups chosen at random were examined internally for anomalies by the method of Monie [17]. This method was chosen since it permits subsequent alizarin red staining of the skeleton and histological study of the viscera.

Data were analyzed by two-way analysis of variance with interactions [18]. The litter was used as the statistical unit. Differences attributable to diet or toxin exposure were considered significant if $P < 0.05$, and those attributable to interaction when $P < 0.10$ [19].

RESULTS

The pregnant protein-malnourished rats gained less weight over the total course of gestation than did those fed the 24% casein diet (Table II). Toxic exposure to 25 mg/kg body weight also reduced weight gain with some interaction between diet and nitrofen exposure. Feed intake was affected when the casein content of the diet was reduced. Although there was a small reduction in intake in animals fed the 8% casein diet, the difference was not statistically significant until the casein level was reduced to 6% or less. Feed efficiency was reduced with each decrease in protein content of the diet. Nitrofen exposure had no significant effect on feed intake or feed efficiency.

Protein deficiency had a striking effect on the number of litters produced. In the case of animals fed the 4% casein diet, in particular, it was necessary to increase markedly the number of females mated since, at low protein intakes, a large number of animals fail to litter [10]. The mean number of young in each litter which was produced ranged from 12.25 to 13.25 and thus did not differ among the various groups. Detailed data on litter size are, therefore, not given.

Since the litter size was unaffected, conceptus death seems to be an all-or-none phenomenon. Nitrofen exposure, by comparison, had little or no effect on the fertility index.

Placental weights were reduced in females fed the protein deficient diets and in those given 25 mg/kg of nitrofen (Table III). There was also an interaction between diet and toxin exposure. As a consequence of the interaction, the reduction in placental weight occurred in dams fed the control

TABLE II

EFFECTS ON THE PREGNANT RAT OF NITROFEN EXPOSURE AND PROTEIN DEPRIVATION

	Diet												Statistical analyses[1]			
	24% casein			8% casein			6% casein			4% casein			*P*			Group differences[2]
	Nitrofen dose, mg/kg body wt.															
	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	D	T	I	
Fertility index[3]	7/8 (87.5%)	7/11 (63.6%)	5/7 (71%)	7/8 (87.5%)	7/8 (87.5%)	5/6 (83.3%)	7/13 (54%)	8/10 (80%)	4/7 (57.1%)	3/13 (23%)	3/15 (20%)	3/19 (15.8%)				
Maternal weight change, g[4]	176.88 ± 7.15(8)[a,c]	163.71 ± 9.75(7)[a]	120.50 ± 8.85(5)[b]	68.57 ± 11.65(7)[c]	67.38 ± 5.94(7)[c]	61.25 ± 10.64(5)[c]	31.14 ± 5.31(7)[d]	31.75 ± 5.38(8)[d]	26.75 ± 8.32(4)[d]	13.00 ± 4.24(3)[d]	11.50 ± 11.50(3)[d]	9.00 ± 3.51(3)[d]	<0.0001	<0.05	<0.02	24/8/6,4(D) O,L/H (T)
Feed intake, g[4]	387.13 ± 12.83(8)	331.50 ± 11.37(7)	333.20 ± 22.94(5)	347.33 ± 21.28(6)	354.43 ± 10.27(7)	315.60 ± 15.96(5)	260.83 ± 21.32(6)	324.13 ± 21.37(8)	307.00 ± 12.28(4)	236.83 ± 12.48(3)	250.75 ± 8.43(3)	282.57 ± 6.02(3)	<0.05	NS	NS	24,8/6,4(D)
Feed efficiency[7]	0.46 ± 0.01(8)	0.40 ± 0.03(7)	0.38 ± 0.01(5)	0.18 ± 0.02(6)	0.19 ± 0.02(7)	0.18 ± 0.02(5)	0.12 ± 0.02(6)	0.11 ± 0.03(8)	0.09 ± 0.03(4)	0.06 ± 0.01(3)	0.08 ± 0.02(3)	0.04 ± 0.01(3)	<0.0001	NS	NS	24/8/6/4(D)

[1] D = diet, T = toxin; I = interaction; 0 = no toxin; L = low dose (12.5 mg/kg); H = high dose (25 mg/kg); NS = not significant.
[2] Slash shows location of significant differences, e.g., 24% and 8% casein diet-fed groups.
[3] Litters produced/number bred.
[4] Total for gestation period.
[5] Mean ± 1 S.E.M.
[6] Values in a row of values with an interaction not bearing a common superscript letter are significantly different.
[7] Gram weight gain/g feed intake.

TABLE III

EFFECTS ON PLACENTAL AND FETAL SIZE OF NITROFEN EXPOSURE IN THE PROTEIN DEPRIVED RAT

	Diet												Statistical analyses[1]			
	24% casein			8% casein			6% casein			4% casein			*P*			Group differences[2]
	Nitrofen dose, mg/kg body wt.															
	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	D	T	I	
Placental weight mg	870.00 ± 50.0(7)[3,4a]	760.00 ± 30.00(7)[b]	636.66 ± 24.16(5)[cd]	600.00 ± 30.0(7)[cd]	620.00 ± 30.0(7)[cd]	590.36 ± 61.88(5)[cde]	474.44 ± 19.47(7)[e]	436.09 ± 14.97(5)[e]	491.56 ± 38.48(4)[de]	463.57 ± 24.92(4)[e]	429.01 ± 23.10(3)[e]	392.44 ± 13.76(3)[e]	<0.0001	<0.02	<0.02	24,8/6,4 O,L/H
Fetal body weight, g	5.42 ± 0.13(8)[a]	5.07 ± 0.11(7)[a]	4.51 ± 0.12(6)[b]	3.83 ± 0.22(7)[c]	4.14 ± 0.12(7)[bc]	3.38 ± 0.19(5)[d]	3.21 ± 0.08(7)[de]	3.21 ± 0.07(5)[de]	3.15 ± 0.25(4)[def]	3.17 ± 0.22(4)[def]	2.81 ± 0.10(3)[ef]	2.52 ± 0.10(3)[f]	<0.0001	<0.0001	<0.05	24/8/6/4 O,L/H
Crown-rump lengh, cm	3.96 ± 0.03(7)[a]	4.00 ± 0.04(6)[a]	3.82 ± 0.04(5)	3.64 ± 0.06(7)	3.79 ± 0.07(8)	3.39 ± 0.12(4)	3.27 ± 0.08(7)	3.34 ± 0.10(5)	3.33 ± 0.10(4)	3.51 ± 0.11(3)	3.26 ± 0.16(3)	3.14 ± 0.07(3)	<0.0001	<0.01	NS	24/8/6,4 O,L/H
Weight/ length	0.75 ± 0.01(7)[a]	0.79 ± 0.01(6)	0.88 ± 0.05(5)	0.95 ± 0.05(7)	0.95 ± 0.02(8)	1.02 ± 0.11(4)	1.04 ± 0.03(7)	1.05 ± 0.02(5)	1.09 ± 0.11(4)	1.20 ± 0.06(3)	1.17 ± 0.07(3)	1.25 ± 0.07(3)	<0.0001	NS	NS	24/8/6,4

[1] D = diet, T = toxin; I = interaction; 0 = no toxin; L = low dose; H = high dose; NS = not significant.
[2] Slash shows location of significant differences among groups.
[3] Mean ± S.E.M.
[4] Values in a row not bearing a common superscript letter are significantly different, $P < 0.05$ or less.

diet, but had no further effect in protein deprived dams. Fetal weight was also significantly reduced with reduction in protein-energy content to 8% or 6%. Fetal weights were also reduced in litters of dams exposed to 25 mg/kg of nitrofen while the other 2 groups did not differ (Table III). Also, diet and nitrofen exposure interacted. When the ratio of placental weight to fetal weight was calculated, the data, not given in the table, showed no effect of either treatment or interaction between diet and toxin.

The length of pups was decreased in comparison to controls in the 8% diet groups and further decreased when casein content was reduced to 6%. Length was also decreased in the fetuses of dams given 25 mg nitrofen/kg body weight. There was no interaction between diet and toxic exposure. The weight per unit length was decreased as dietary protein was reduced, but there was no toxic effect.

The weights of all organs studied were decreased as a consequence of the reduction in protein-calorie intake (Table IV). However, not all organs were significantly affected at the same protein-calorie level. Heart weight in fetuses of protein-deprived dams did not differ from the controls until the protein level was reduced to at least 6%. All other organs were reduced in weight when the 8% casein diet was fed to the dams. In the intestine, lung and brain, a second reduction in size occurred in young of dams fed the 6% casein diet, and in the liver and kidney, when the 4% casein diet was fed. A third reduction occurred in the intestine and brain in the 4% casein group.

Nitrofen exposure affected lung size in the lower dose group with no further effect with the higher dose. Liver, kidney, intestine, heart and brain weights were reduced in the higher dose group but were unaffected by the lower dose. An interaction between nutritional deficiency and nitrofen exposure affected the kidneys, intestine and lung. Kidney weights tended to decrease with progressively lower casein content of the diet and increasing nitrofen dosage, while in the intestine and lungs, it appears that nitrofen exposure had a greater effect on fetal organ weight in the 24% and 8% casein-fed groups than in the more restricted groups.

When organ weight-body weight ratios were analyzed (Table V), the data indicated that liver, kidney, and heart were a decreased proportion of the body weight in fetuses of groups fed any of the protein-calorie deficient diets, while diet did not decrease the intestinal weight until the protein content was reduced to 4%. Relative lung weights were unaffected by diet. The proportion of body weight which represented brain and heart weights increased as dietary casein content was reduced.

Toxic effects were seen in relative intestine and lung weights when the dams were given 25 mg nitrofen/kg. The lower dose was intermediate between the other 2 groups and did not differ from either. Relative liver, kidney, heart, and brain weights were unaffected by toxin exposure alone. In the kidney, intestine and brain, the data also suggest an effect from an interaction between the deficient diet and toxic exposure. In the kidney and intestine, significant difference from controls existed only when the most restricted diet was combined with the highest nitrofen dose. In the

TABLE IV

EFFECTS ON FETAL ORGAN WEIGHTS OF NITROFEN EXPOSURE AND PROTEIN DEFICIENCY IN THE PREGNANT RAT

	Diet												Statistical analyses[1]			
	24% casein			8% casein			6% casein			4% casein			*P*			Group differences[2]
	Nitrofen dose, mg/kg body wt.															
	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	D	T	I	
Liver, mg	422.28 ± 10.29(7)[3]	392.93 ± 21.45(6)	342.54 ± 28.51(5)	266.79 ± 17.47(7)	274.74 ± 19.55(7)	188.57 ± 13.02(4)	224.96 ± 10.18(7)	266.87 ± 20.88(8)	208.40 ± 25.62(4)	182.40 ± 6.82(3)	192.85 ± 7.72(3)	161.93 ± 16.22(3)	<0.0001	<0.002	NS	24/8 6/4 O,L/H
Kidney, mg	57.45 ± 2.52(7)[a,a]	46.05 ± 2.36(6)[b]	45.15 ± 4.13(5)[b]	37.78 ± 2.23(7)[c]	37.87 ± 2.11(7)[c]	28.64 ± 4.05(4)[de]	29.61 ± 1.39(7)[de]	29.82 ± 2.14(8)[de]	29.30 ± 4.49(4)[de]	23.71 ± 0.87(3)[de]	24.24 ± 2.75(3)[d]	20.03 ± 1.72(3)[e]	<0.0001	<0.01	<0.09	24/8,6/4 O,L/H
Intestine, mg	203.55 ± 9.40(7)[a]	182.51 ± 9.20(6)[a]	138.28 ± 8.84(5)[bc]	136.23 ± 10.26(7)[bc]	143.36 ± 8.17(7)[b]	104.48 ± 10.60(4)[def]	108.44 ± 3.79(7)[de]	119.59 ± 6.66(8)[cd]	112.16 ± 11.00(4)[de]	86.27 ± 2.59(3)	84.61 ± 6.77(3)[ef]	74.14 ± 8.62(3)[f]	<0.0001	<0.001	<0.02	24/8/6/4 O,L/H
Heart, mg	46.67 ± 2.15(7)	48.42 ± 3.69(6)	38.58 ± 5.46(5)	46.11 ± 3.04(7)	46.25 ± 2.15(7)	29.58 ± 1.52(4)	37.81 ± 0.83(7)	37.49 ± 2.35(8)	30.80 ± 7.07(4)	34.06 ± 1.95(3)	29.56 ± 3.90(3)	27.43 ± 1.35(3)	<0.0001	<0.002	NS	24,8/6,4 O,L/H
Lung, mg	138.06 ± 6.94(7)[a]	108.35 ± 3.21(6)[b]	99.71 ± 3.69[bc]	101.70 ± 8.40(7)[bc]	90.18 ± 4.93[cd]	87.84 ± 7.42(4)[cde]	72.60 ± 3.79(7)[ef]	77.81 ± 6.48(8)[ef]	73.05 ± 3.56(4)[ef]	67.64 ± 4.25(3)[ef]	66.15 ± 5.02(3)[ef]	63.37 ± 11.48(3)[f]	<0.0001	<0.005	<0.05	24/8/6,4 O/L,H
Brain, mg	210.74 ± 2.66(7)	214.92 ± 2.09(6)	192.84 ± 6.66(5)	188.26 ± 3.72(7)	201.29 ± 3.09(7)	181.20 ± 7.10(4)	177.86 ± 4.18(7)	181.61 ± 2.68(8)	178.27 ± 6.04(4)	170.49 ± 2.17(3)	170.57 ± 8.71(3)	160.68 ± 4.53(3)	<0.0001	<0.0002	NS	24/8/6/4 O,L/H

[1] D = diet, T = toxin; I = interaction; 0 = no toxin; L = low dose; H = high dose; NS = not significant.
[2] Slash shows location of differences among groups.
[3] Mean ± 1 S.E.M.
[4] Values in a row not bearing a common superscript letter are significantly different, $P < 0.05$ or less.

TABLE V

EFFECTS ON RELATIVE FETAL ORGAN WEIGHTS OF NITROFEN EXPOSURE AND PROTEIN DEFICIENCY IN THE PREGNANT RAT

	Diet												Statistical analyses[1]			
	24% casein			8% casein			6% casein			4% casein			*P*			Group differences[2]
	Nitrofen dose, mg/kg body wt.															
	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	0	12.5	25.0	D	T	I	
Liver	7.89 ± 0.15(7)[3]	8.04 ± 0.54(6)	7.92 ± 0.51(5)	6.96 ± 0.41(7)	7.10 ± 0.34(7)	5.42 ± 0.12(4)	7.47 ± 0.73(3)	6.47 ± 0.36	7.00 ± 0.64(4)	6.49 ± 0.45(2)	6.92 ± 0.38(2)	6.07 ± 0.56(3)	<0.001	NS	NS	24/8,6,4
Kidney	1.07 0.044 (7)[4,a]	0.934 ± 0.023 (6)[abcd]	1.050 ± 0.065 (5)[abc]	0.982 ± 0.029 (7)[abc]	0.986 ± 0.072 (7)[abc]	0.799 ± 0.081 (4)[cd]	0.998 ± 0.094[bc]	0.858 ± 0.029[bcd]	0.988 ± 0.125 (4)[abc]	0.847 ± 0.021 (2)[bcd]	0.943 ± 0.075 (2)[abcd]	0.759 ± 0.056 (3)[d]	<0.02	NS	<0.09	24/8,6,4
Intestine	3.77 ± 0.135 (7)[a]	3.74 ± 0.069 (6)[ab]	3.22 ± 0.100 (5)[abc]	3.54 ± 0.180 (7)[ab]	3.71 ± 0.130 (7)[ab]	3.00 ± 0.200 (4)[c]	3.77 ± 0.200 (3)[ab]	3.46 ± 0.140 (8)[ab]	3.82 ± 0.340 (4)[a]	3.07 ± 0.127 (2)[b]	3.14 ± 0.346[abc]	2.91 ± 0.300 (3)[c]	<0.01	<0.05	<0.10	24,8,6/4 O,L/L,H
Heart	0.87 ± 0.056(7)	0.964 ± 0.047(6)	0.890 ± 0.098(5)	1.22 ± 0.13(7)	1.22 ± 0.092(7)	0.855 ± 0.042(4)	1.210 ± 0.31(3)	1.093 ± 0.07(8)	1.03 ± 0.16(4)	1.245 ± 0.155(2)	1.14 ± 0.165(2)	1.048 ± 0.049(3)	<0.02	NS	NS	24/8,6,4
Lung	2.57 ± 0.12(7)	2.24 ± 0.078(6)	2.32 ± 0.044(5)	2.63 ± 0.12(7)	2.36 ± 0.17(7)	2.53 ± 0.16(4)	2.29 ± 0.19(3)	2.23 ± 0.08(8)	2.52 ± 0.16(4)	2.37 ± 0.126(2)	2.22 ± 0.45	2.23 ± 0.27(3)	NS	<0.05	NS	O,L/L,H
Brain	3.92 ± 0.086 (7)[a]	4.30 ± 0.13 (6)[b]	4.54 ± 0.10 (5)[abc]	4.95 ± 0.20 (7)[bc]	5.26 ± 0.17 (7)[c]	5.25 ± 0.184 (4)[c]	6.01 ± 0.41 (3)[d]	5.35 ± 0.23 (8)[c]	6.13 ± 0.27 (4)[d]	6.29 ± 0.034 (2)[d]	6.30 ± 0.42 (2)[d]	6.16 ± 0.22 (3)[d]	<0.0001	NS	<0.086	24/8/6,4

[1] D = diet; T = toxin; I = interaction; 0 = no toxin; L = low dose; H = high dose; NS = not significant.
[2] Slash shows location of differences among groups.
[3] Mean ± 1 S.E.M.
[4] Values in a row not bearing a common superscript letter are significantly different.

TABLE VI

CONGENITAL ANOMALIES IN FETUSES OF RATS MALNOURISHED AND EXPOSED TO NITROFEN DURING GESTATION

Diet % casein	Nitrofen (mg/kg body wt)	Anomalies		Affected litters (%)
		Diaphragmatic hernia	Other	
24	0	0	0	0
	12.5	6 (4)[1]	1[2]	51
	25	7 (3)	0	60
8	0	0	1[3]	14
	12.5	5 (4)	3 (2)[4,5]	51
	25	3 (2)	2 (1)[6]	40
6	0	0	0	0
	12.5	3 (3)	1[4]	43
	25	2 (2)	1[7]	50
4	0	0	0	0
	12.5	1 (1)	0	33
	25	0	1[8]	33

[1] Number of affected pups (number of litters).
[2] Nostrils not open.
[3] Cataracts.
[4] One enlarged ventricle.
[5] Two ectopic kidneys in 1 litter.
[6] Hydroureter.
[7] Missing lobe of lung.
[8] Hemorrhagic lungs.

brain, the increased proportion of the brain relative to body weight seen in the young of control diet-fed animals exposed to nitrofen was not maintained in the protein-deprived groups.

Congenital anomalies observed are listed in Table VI. There was only one anomaly in animals not exposed to nitrofen. In nitrofen-exposed animals, diaphragmatic hernia was the anomaly most frequently seen.

There were no differences in numbers of ossification sites. There were no differences attributable to location in the uterus or fetal sex. Therefore, data for these parameters are not given.

DISCUSSION

Since nutritional deficiencies commonly result in a decrease in food intake, a diet which is deficient in protein usually results in a protein-energy deficient intake. As the food intake decreases, protein is used as a source of energy, thus exacerbating the protein deficiency. Thus the diet used in this

study is best described as protein-energy deficient. Animals pair-fed the control diet in amounts eaten by protein-deprived dams have been used to identify those results attributable to reduced calorie intake. However, the 2 deficiencies generally do not occur separately. In human nutrition, as it usually occurs, for example, protein and energy malnutrition are combined. Therefore, separation of the 2 deficits was not done in this work.

Controversy exists concerning the relative effects of protein-energy malnutrition on the maternal body and on the fetus. There is, on the one hand, the school of thought which states that the fetus is a parasite at the expense of the mother [20,21]. On the other hand, there is the belief that the fetus is affected. It has been shown in animals [10—16,22] and in humans [23] that maternal protein-calorie malnutrition can affect the fetus. Even if we accept that the fetus is not a perfect parasite, the proportion of the effect on the fetus and on the mother remains a matter of debate [24,25]. In addition, the mechanisms modulating nutrient partition between mother and fetus are unclear.

The data obtained in this work support the concept that malnutrition can affect the mother, as demonstrated by the decreased fertility index and reduced maternal weight gain. In addition, it has been shown previously that the pregnant rat adapts to pregnancy by increasing its feed efficiency and that chronic malnutrition in pregnancy prevents this adaptation [26]. The present work demonstrates that this adaptation failure also occurs in more acute protein-calorie malnutrition.

The fact that malnutrition also affects the conceptus is demonstrated by reduced placental weight, reduced fetal weight and length, and reduction in sizes of various organs. The reduction in organ size, as shown previously [10], is not always proportional to the reduced body size. The fetal brain and heart have been shown to be conserved while the kidney is more affected by diet.

The reduced fetal size and incidence of diaphragmatic hernias are compatible with previous studies of the effects of similar levels of nitrofen exposure during pregnancy [5,27]. Studies of organ sizes have not been done previously; therefore comparison with values in the literature is not possible.

The range of effects of toxic exposure on the fetus has been classified in 5 categories: (1) conceptus death; (2) production of major terata; (3) increased incidence of minor anomalies; (4) overall growth retardation; and (5) no observable effects [27]. There is no evidence that nitrofen causes death of conceptuses in utero since litter sizes did not differ, although a high postnatal mortality rate has been reported [28]. Also, when the fertility index is averaged across diet, there is not a marked difference (63% in non-exposed and low dose groups and 57% in the high dose group). In addition, it appears that maternal nitrofen exposure did not produce major terata visible with the methods used. Thus, categories 1 and 2 above might be eliminated. The biological significance of the third and fourth categories above are unclear. Kavlock et al. [27] have suggested that together they be considered to be examples of "fetotoxicity". The effects of nitrofen

exposure on the conceptuses in this study appear to be appropriately categorized as fetotoxic since the reduction in size indicates growth retardation. In addition, in examinations such as those used in this work, anomalies might be classified as minor.

A problem arises, however, from the fact that, although gross anomalies are not visible, pups whose dams are given 25 mg/kg of nitrofen will, for the large part, die of some teratologic event [5]. Nitrofen is a somewhat unique teratogen in this regard causing neonatal death in the absence of observable defects. Although fewer total anomalies were produced in the 4% casein diet, high nitrofen dose group, this may be a function of the small number of surviving litters.

Some suggestion as to the possible biological significance of effects of nitrofen exposure on pups surviving to term may be obtained when individual organs are considered in relation to body weight. In the case of liver, kidney, intestine, heart, and lung, a case could be made that smaller organs in smaller pups could have little biological significance, provided the organs are as functionally mature as are the groups not exposed to nitrofen. Examination of kidney and intestine morphology and function in young of protein-deprived dams previously has shown that these smaller organs are immature [13,29]. The liver, heart and lung were not studied in similar fashion. It might be speculated that maternal nitrofen exposure results in more marked immaturity in the young, at least in proportion to the decrease in body weight. In the intestine and lung, however, the higher dose apparently also has a specific organ toxicity and, in the intestine and kidney, an interaction with the diet. The data thus suggest a greater effect on the intestine, kidney and lung than on the liver or heart. Studies of the specific effects on morphology and function of these three organs are underway.

Kavlock et al. [9] report a high incidence of hydronephrosis in young of nitrofen-exposed dams. The methods of examination in the present study would detect hydroureter but not hydronephrosis. Therefore, the results of the 2 papers are not contradictory.

The significance of the differences in brain weights are difficult to interpret. Clearly, brain weight is reduced as a consequence of the diet restriction. However, as shown in this paper, as well as previously [10], brain weight is conserved in relation to body weight. The influence of differences in brain size on central nervous system function has been a matter of controversy for decades, and thus, the significance of the observed interaction between diet and nitrofen exposure is difficult to interpret. A study of brain biochemistry and morphology, as well as postnatal nervous system development would be useful to this end.

Overall, the data indicate little interaction between marginal maternal protein-energy deficit and the lower nitrofen dose. Some evidence of interaction between maternal protein-energy deficiency and maternal nitrofen exposure, however, occurs with severe malnutrition and the higher toxic dose. These results do not explain the previously reported high postnatal mortality rate in the offspring, but may suggest some internal organs whose functions might be investigated.

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